

**Detailed Action**

***Status of Application, Amendments, And/Or Claims:***

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submissions, filed on 7 September 2010 and 10 May 2010, have been entered.

Applicants have also submitted a Declaration by Professors Olli Vuolteenaho and Heikki Ruskoaho under 35 USC § 1.132 on 10 May 2011. This declaration will be addressed below.

Claims 1-4, 7-10, 12-17, 29-37, 40-44, and 62-68 are pending in the instant application. Claims 29-37 and 40-44 stand withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. In the original response to requirement for election of species (Response of 10 July 2008), applicants elected the following species: SEQ ID NO:3 (NT-proANP), SEQ ID NO:6 (NT-proBNP), SEQ ID NO:9 (polynucleotide encoding SEQ ID NO:3), and SEQ ID NO:12 (polynucleotide encoding SEQ ID NO:6).

Claims 1-4, 7-10, 12-17, and 62-68 are under consideration to the extent they read on the elected invention.

**Withdrawn Objections/Rejections**

Claims 11, 18-21, 23-27, 46-52, 59 and 60 are canceled; all objections and rejections of these claims are thereby moot.

The rejection of Claims 2, 3, 10, and 12 under 35 U.S.C. 112, second paragraph as vague and indefinite in reciting "a fusion polypeptide agent or a fusion peptide agent" is withdrawn. Applicants have amended the claims to delete "or a fusion peptide agent", thereby obviating the rejection.

The rejection of Claims 2 and 3 under 35 U.S.C. 112, second paragraph as vague and indefinite for reciting duplicate limitations is withdrawn in light of Applicants' amendment to the claims.

The rejection of Claim 3 under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps is withdrawn upon further consideration, and in light of Applicants' persuasive argument.

The rejection of Claims 1-4, 7-10, 12-17 and 62-68 under 35 U.S.C. 112, first paragraph, because the specification does not reasonably provide enablement for a method comprising contacting the sample with a bi- or oligospecific first binding substance wherein said binding substance is an antibody or antigen binding fragment thereof, said binding substance being able to bind to a fragment of (a) or (b) which is at least 6 amino acids in length is withdrawn in light of Applicants' amendment to the claims.

The rejection of Claims 1-4, 7-10, 12-17 and 62-68 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is withdrawn in light of Applicants' amendment to the claims.

#### **Maintained and/or New Rejections**

##### ***35 U.S.C. § 112, Second Paragraph:***

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-4, 7-10, 12-17, and 62-68 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1, the independent claim of the instant invention is vague and indefinite in reciting "detecting in a single reading, in a single assay the presence of atrial and brain natriuretic peptide prohormones (proANP and proBNP) or fragments thereof in a sample...". The claim reads on detecting the presence of proANP and proBNP;

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however, it is unclear how one can detect the presence of these proteins without detecting the presence of each one of these proteins individually. If applicants intend to detect the sum of proANP and proBNP, the claim should be amended to indicate that such is applicants' invention. The claim is also vague and indefinite in reciting "a reference level". It is unclear what the reference level is; is the reference level the level of each hormone that is considered "normal" or "baseline" level or the "normal" or "baseline" level of the sum of these two prohormones? Furthermore, it is unclear how one is to detect "activation" or "inactivation" of each of the ANP and BNP systems without detecting the presence of proANP and proBNP fragments individually.

Claims 12 and 14 are vague and indefinite in reciting "and/or"; a recitation in the alternative renders the claims indefinite.

Claim 17 is vague and indefinite in reciting "wherein detection of activation of the ANP and BNP hormonal systems is diagnostic of heart failure, or detection of inactivation of ANP and BNP hormonal systems monitors successful treatment of a cardiac condition". It is unclear how one is to determine activation or inactivation of ANP or BNP hormonal systems if one cannot detect levels of the individual pro-hormones.

The remainder of the claims is included in the rejection as dependent upon a rejected claim.

### ***35 U.S.C. § 112, First Paragraph***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

### ***Scope of Enablement***

Claims 1, 2, 7, and 8, are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of determining activation of the ANP and BNP hormonal system said method comprising detecting in a single reading, in a single assay the presence of atrial and brain natriuretic peptide prohormones or fragments thereof which comprises:

Contacting the sample with a bi-or oligospecific first binding substance wherein said binding substance is an antibody or antigen binding fragment or derivative thereof

does not reasonably provide enablement for a method comprising

Contacting the sample with a bi-or oligospecific first binding substance wherein said binding substance is an antibody fragment or derivative.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

The factors considered when determining if the disclosure satisfies the enablement requirement and whether any necessary experimentation is undue include, but are not limited to: 1) nature of the invention, 2) state of the prior art, 3) relative skill of those in the art, 4) level of predictability, 5) existence of working examples, 6) breadth of claims, 7) amount of direction or guidance by the inventor, and 8) quantity of experimentation needed to make or use the invention. *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

The claims are broadly drawn to a method comprising contacting the sample with a binding substance (antibody) or a fragment or derivative thereof. The claims do not require that the antibody fragment or derivative retain the property of binding to proANP and proBNP. The full scope of the claims is not enabled for the following reasons.

The specification teaches detection methods utilizing antibodies which recognize both A- and B-type natriuretic peptide prohormones and discloses conventional immunoassays which are well known in the art [paragraph 0144 of PGPUB 20070141634, the PGPUB of the instant invention]. The disclosure teaches "An antibody according to the invention may comprise either a whole antibody or a fragment

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thereof and has the binding specificity set out above (i.e., "specifically binds" to a polypeptide when it binds with preferential or high affinity to the protein or proteins for which it is specific but does substantially not bind or binds with only low affinity to other polypeptides [paragraph 0196]) [paragraph 0197]. A fragment of whole antibody that can be used in the method comprises an antigen binding site, e.g. F(ab') or F(ab)<sub>2</sub> fragments. Such fragments or antibodies may be used to form antibody derivatives" [paragraph 0199]. The working examples teach utilization of polyclonal goat antibody based binding substances to recognize NT-proXNP, NT-proANP and NT-proBNP simultaneously [paragraph 0315]. There are no examples, working or prophetic, utilizing antibody fragments or derivatives in the methods of the instant invention.

Thus, the specification does not teach or provide guidance or working examples as to how to use a variant antibody (fragment or derivative) without a functional property.

The specification provides no guidance or working examples as to how the skilled artisan could use inactive variants of the claimed antibody, as no functional limitation is associated with the variants in the claims. Accordingly, the specification neither provides adequate guidance as to how to *make* functional species, nor how to *use* those that are not, which would be expected to be the majority of species.

The state of the prior art is such that it is well established in the art that the formation of an intact antigen binding site of antibodies routinely requires the association of the complete heavy and light chain variable regions each of which consists of three CDRs or hypervariable regions, which provide the majority of the contact residues for the binding of the antibody to its target epitope (Paul, *Fundamental Immunology*, 3<sup>rd</sup> Edition, 1993, pp. 292-295, under the heading "Fv Structure and Diversity in Three Dimensions"). The amino acid sequences and conformations of each of the heavy and light chain CDRs are critical in maintaining the antigen binding specificity and affinity, which is characteristic of the immunoglobulin. It is expected that all of the heavy and light chain CDRs in their proper order and in the context of framework sequences which maintain their required conformation, are required in order to produce a protein having antigen-binding function and that proper association of heavy and light chain variable regions is required in order to form functional

antigen binding sites (Paul, page 293, first column, lines 3-8 and line 31 to column 2, line 9 and lines 27-30). Even minor changes in the amino acid sequences of the heavy and light variable regions, particularly in the CDRs, may dramatically affect antigen-binding function as evidenced by Rudikoff et al (Proc. Natl. Acad. Sci. USA, 79(6):1979-1983, March 1982). Rudikoff et al. teach that the alteration of a single amino acid in the CDR of a phosphocholine-binding myeloma protein resulted in the loss of antigen-binding function. Colman P. M. (Research in Immunology, 145:33-36, 1994) teaches that even a very conservative substitution may abolish binding or may have very little effect on the binding affinity (see pg. 35, top of left column and pg. 33, right column).

It would require undue experimentation to prepare the myriad of antibody fragments or derivatives that meet the limitations recited in the claims, and determine which of said variants exhibit the requisite biological activities.

Due to the large quantity of experimentation necessary to generate the myriad of antibody fragments or derivatives that meet the limitations recited in the claims, and to determine how to use the said fragments or derivatives, the lack of direction/guidance presented in the specification regarding same, the absence of working examples directed to same, and the breadth of the claims which embrace a broad class of structurally diverse fragments or derivatives without a meaningful functional limitation, undue experimentation would be required of the skilled artisan to make and/or use the claimed invention in its full scope.

***35 U.S.C. § 103:***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

- (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

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The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

The rejection of Claims 1, 16, 17, 62-66 and 68 under 35 U.S.C. 103(a) as being unpatentable over Clerico et al (1998. J Endoc. Invest 21:170-179), in view of Clerico et al. (2000. Clin. Chemistry 46:1529-1534) is maintained for reasons of record and for reasons set forth below.

The claims are drawn to an *in vitro* method comprising simultaneously detecting, in a single reading, in a single assay, the presence of atrial and brain natriuretic prohormones (proANP and proBNP) in a sample, wherein the method comprises an immunoassay (Claim 16), thereby diagnosing heart failure or monitoring treatment of a cardiac condition (Claim 17), wherein said reference level is determined from a previous measurement from said subject (Claim 62), wherein said reference level is based on the normal level of a population of subjects (Claim 63), wherein said population of subjects is the general population (Claim 64), wherein said assay is calibrated so that a particular reading in the assay is known to represent the normal peptide level (Claim 65), wherein

said assay is calibrated so that a normal level will produce a negligible or insignificant result (Claim 66) wherein said subject is a human (Claim 68).

Clerico et al (1998) teach measurement of plasma atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels in plasma of patients with heart failure as an assay method useful in follow-up of cardiac patients (monitoring a cardiac condition) (abstract). The measurements are performed on plasma samples from healthy human subjects and patients with chronic cardiomyopathy (page 172, 1<sup>st</sup> column, 2<sup>nd</sup> paragraph). The measurements from healthy, normal subjects in the general population were used to establish a reference level of normal ANP and normal BNP levels (Figure 3). Both polypeptides were measured in samples from the same subject (page 174, 1<sup>st</sup> column, 3<sup>rd</sup> paragraph and page 175, 2<sup>nd</sup> column, last paragraph). The measurements were performed using non-competitive immunoradiometric assays (IRMA) (page 172, 1<sup>st</sup> column, last paragraph bridging page 173, 2<sup>nd</sup> column, 1<sup>st</sup> paragraph). The reference teaches utilizing standard solutions comprising known quantities of ANP and BNP to generate standard curves which act as reference values to determine the amount of ANP and BNP in the samples from patient subject (Page 172, 2<sup>nd</sup> column, 2<sup>nd</sup> paragraph and page 173, 1<sup>st</sup> column, 1<sup>st</sup> paragraph). While the reference does not teach determining a reference level from a previous measurement from the same subject, the reference does teach that the assay methods for these peptides may be useful in the follow-up of cardiac patients, thus teaching the advantage of comparing the detected level of the natriuretic peptides in one assay to those detected in a sample from the same patient in a previously performed assay.

Clerico et al (1998) does not teach a method comprising detecting the presence of atrial and brain natriuretic peptide prohormones or fragments thereof. Clerico et al (2000) teach that cardiac natriuretic hormones are a family of related peptides including ANP, BNP and other peptides derived from the N-terminal portion of proANP and proBNP peptide chains (abstract). The reference teaches that the N-terminal prohormones (NT-proANP and NT-proBNP) are present in greater amounts in the plasma than ANP and BNP (Table 1).

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the methods taught by Clerico et al (1998) and substitute measurement of proANP and proBNP (as taught by Clerico et al (2000)) for the measurement of ANP and BNP as taught by Clerico et al. (1998). The person of ordinary skill in the art would have been motivated to make these modifications because Clerico et al (2000) teach that the prohormones are present in higher concentrations in the plasma and one of ordinary skill in the art would recognize that these may be measured more easily and accurately. One would reasonably have expected success because methods of measuring said prohormones are outlined by Clerico et al (2000).

Additionally, one of ordinary skill, aware that it is routine to detect multiple compounds in a single sample at the same time in the performance of clinical assays (for example, a lipid profile, liver enzyme assays), would be motivated to assay both ANP and BNP in the same assay to increase the efficiency and reduce the costs of said assays. Techniques utilizing immunoassays for simultaneous detection of two polypeptides in a single reading in a single assay were well known at the time of the instant invention, as evidenced by Swartzman et al which teaches simultaneous detection of two cytokines, IL-6 and IL-8 in the same high-throughput multiplexed immunoassay. (See, for evidentiary purposes only, Swartzman et al. 1999. Analytical Biochem. 271:143-151, abstract)

Absent evidence that assaying for one protein, i.e. detection of ANP, would interfere with the detection of the second protein, i.e. detection of BNP, one of ordinary skill would anticipate success in detecting both proteins simultaneously in the same sample.

With respect to the limitations recited in Claims 65 and 66. While the references do not teach assays calibrated so that a particular reading in the assay is known to represent the normal peptide level (Claim 65) or assays wherein the assay is calibrated so that a normal level will produce a negligible or insignificant result (Claim 66), one of ordinary skill in the art is aware that calibration of read-out instruments, such as FACs machines or counters which detect radioactivity, to set base-line levels at pre-determined, desired levels is routine in the art of immunoassays.

Applicants traverse the rejection (Response of 10 May 2011, page 2, 3<sup>rd</sup> paragraph, bridging page 3, 2nd paragraph).

Applicants have submitted a declaration under 35 U.S.C. § 1.132 by Professors O. Vuolteenaho and Ruskohao (10 May 2011). The declaration asserts:

(Section 4) There is significant basal secretion of proANP-derived peptides, whereas proBNP- derived peptides are formed and secreted on demand. Moreover, BNP has a lower affinity to NPR-A as compared to ANP. Thus, small absolute increases of BNP do not induce significant biological effects. A pure BNP or N-terminal fragment of pro-BNP (NT-proBNP) assay detects them as proportionally large increases, because of the negligible basal secretion of proBNP peptides. On the other hand, relatively small proportional increases of proANP-derived peptides induce marked biological effects. Concomitant increase of both proANP- and proBNP-derived peptides, even when modest, indicates a major pathophysiological induction of the cardiac natriuretic peptides.

(Section 5) Measurement of proANP- and proBNP-derived peptides with separate assays requires the optimization, calibration, and maintenance of at least two immunoassays, with resulting technical complexity and high cost. Use of two assays also requires sophisticated software for extraction of clinically relevant information from the non-linear data from the two assays. The results from the separate assays reported to the physician would then consist of two analytical values and their mathematical interpretation, and would not be very easily understandable or intuitive. Thus, routine measurement of proANP- and proBNP-derived peptides is not a practical or viable alternative for routine clinical application.

(Section 6) We devised a novel method which detects activation or inactivation of the ANP and BNP hormonal systems by assaying for both proANP- and proBNP- derived peptides in the same sample, at the same time, in a single reading, in a single assay. ... it appears to circumvent the above- described pitfalls associated with the use of separate assays, because it mimics more closely the way the body processes the biological information carried by the cardiac natriuretic peptides. The method produces a single result, does not require sophisticated data extraction, and is simpler to perform

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than prior art methods. Small increases of only NT-proBNP are masked by the high basal levels of NT-proANP, thus decreasing the risk of false positive results. On the other hand, even small increases of both NT-proANP and NT-proBNP can be detected with high sensitivity, thus decreasing the risk of false negative results. Thus, the technical innovation of the present invention provides a superior clinical method without increasing the technical complexity or cost.

(Section 7) A practical example in Figure 4/5 of the present application, measures clinical samples of 500 patients with heart failure. Unexpectedly the NT-proANP value (Figure 4/5, bar on the right) provides a clearly better separation between the different New York Heart Association (NYHA) classes, and thus has more clinical power, as compared to NT- proANP alone (bar on the left), NT-proBNP alone (bar in the middle), or their arithmetic sum, measured from the same samples.

Applicant's arguments have been fully considered but are not found to be persuasive for the following reasons:

In assessing the weight to be given expert testimony, the examiner may properly consider, among other things, the nature of the fact sought to be established, the strength of any opposing evidence, the interest of the expert in the outcome of the case, and the presence or absence of factual support for the expert's opinion. See Ex parte Simpson, 61 USPQ2d 1009 (BPAI 2001), Cf. Redac Int'l. Ltd. v. Lotus Development Corp., 81 F.3d 1576, 38 USPQ2d 1665 (Fed. Cir. 1996), Paragon Podiatry Lab., Inc. v. KLM Lab., Inc., 948 F.2d 1182, 25 USPQ2d 1561, (Fed. Cir. 1993).

Applicants have sought to overcome the rejection of Claims 1, 16, 17, 62-66 and 68 under 35 U.S.C. 103(a) as being unpatentable over Clerico et al (1998. J Endoc. Invest 21:170-179), in view of Clerico et al. (2000. Clin. Chemistry 46:1529-1534), which teach detection of each of these pro-hormones in individual assays and teach that measurement of plasma atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels in plasma of patients with heart failure is useful in monitoring efficacy of therapy in cardiac patients.

In section 4 of the affidavit, Applicants argue that there is a significant difference in basal secretion or proANP-derived peptides and BNP-derived peptides and that small

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absolute increases of BNP do not induce significant biological effects. However, relatively small proportional increases of proANP-derived peptides induce marked biological effects. "Concomitant increase of both proANP- and proBNP-derived peptides, even when modest, indicates a major pathophysiological induction of the cardiac natriuretic peptides". However, Applicants' statements appear to contradict this conclusion. Based on the statements in section 4, one of ordinary skill would conclude that it would be advantageous to measure proANP-derived peptides and BNP-derived peptides individually. It would appear that a biologically insignificant (but statistically significant) increase in BNP-derived peptides would swamp out any real indication of biologically significant increases in the two peptides. One would conclude that increases in pro-ANP would provide more information as to the physiological state of the patient.

In sections 5 and 6, applicants argue the technical and economic advantages of a single assay compared to individual assays for proANP- and proBNP-derived peptides. These are secondary considerations. Evidence pertaining to secondary considerations must be taken into account whenever present; however, it does not necessarily control the obviousness conclusion. See, e.g., *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1372, 82 USPQ2d 1321, 1339 (Fed. Cir. 2007); *Leapfrog Enterprises Inc. v. Fisher-Price Inc.*, 485 F.3d 1157, 1162, 82 USPQ2d 1687, 1692 (Fed. Cir. 2007) ("given the strength of the *prima facie* obviousness showing, the evidence on secondary considerations was inadequate to overcome a final conclusion" of obviousness); and *Newell Cos., Inc. v. Kenney Mfg. Co.*, 864 F.2d 757, 768, 9 USPQ2d 1417, 1426 (Fed. Cir. 1988). (See MPEP § 2145). Additionally, "The fact that a combination would not be made by businessmen for economic reasons does not mean that a person of ordinary skill in the art would not make the combination because of some technological incompatibility. *In re Farrenkopf*, 713 F.2d 714, 219 USPQ 1 (Fed. Cir. 1983) (Prior art reference taught that addition of inhibitors to radioimmunoassay is the most convenient, but costliest solution to stability problem. The court held that the additional expense associated with the addition of inhibitors would not discourage one

of ordinary skill in the art from seeking the convenience expected therefrom.) (See MPEP § 2145). Thus, the technical advantages argued by applicants is not sufficient to overcome the outstanding rejection.

In section 7, Applicants argue that Figure 4 indicates unexpected results: "Unexpectedly the NT-proXNP value (Figure 4/5, bar on the right) provides a clearly better separation between the different New York Heart Association (NYHA) classes, and thus has more clinical power, as compared to NT- proANP alone (bar on the left), NT-proBNP alone (bar in the middle), or their arithmetic sum, measured from the same samples. However, Applicants arguments are not persuasive. If one examines the data presented in Figure 4, the level of detected NT-proXNP in patients in NYHA 1-3 classes is identical to the level of detected NT-proANP; thus it is unclear how detecting NT-proXNP provides more "clinical power" than detecting NT-proANP itself. With respect to NYHA 4, it is unclear if the level of detected NT-proXNP is statistically different from the level of detected pro-ANP, as applicants have provided a graph with only error bars and have not provided the data itself. The pattern of increases of all three markers is the same as one looks at the patients in all four categories; thus it is unclear how the pattern of increase in NT-proXNP is an unexpected result.

The rejection is thus maintained.

The rejection of Claims 2-4, 7-10, 12-15 and 67 under 35 U.S.C. 103(a) as being unpatentable over Clerico et al (1998) in view of Clerico et al. (2000) as applied to claim 1 further in view of Buechler et al (US 7,341,838, filed 19 April 2004, priority claimed to provisional application 60/466,358, filed 28 April 2003, the '838 patent) is maintained for reasons of record and for reasons set forth below.

The teachings of Clerico et al (1998) and Clerico et al (2000) are outlined in detail above. In addition to the teachings above, Clerico et al. (2000) teach competitive assays such as radioimmunoassays comprising labeled antigens such as ANP and BNP (abstract).

The references, singly or in combination, do not teach the following further limitations:

contacting the sample with a bi- or oligo-specific binding substance that is able to bind to both NT-proANP of SEQ ID NO:3 and NT-proBNP of SEQ ID NO:6 and a fusion polypeptide (Claims 2 and 3),

wherein said fusion polypeptide agent is used as a calibration agent or competitive inhibitor (claims 3 and 67)

wherein the fusion polypeptide comprises pro-BNP1-76 (SEQ ID NO:6) and proANP 1-98 (SEQ ID NO:3) (claims 9 and 10),

wherein the binding substance comprises a bi- or oligo specific binding substance or a mixture of mono-specific binding substances (Claim 4), an antibody that binds to NT-proANP of SEQ ID NO:3 and NT-proBNP (SEQ ID NO:6) (Claims 7 and 8), wherein the first binding substance or agent is labeled and/or immobilized (claim 12) and a method which additionally comprises contacting the sample with a second binding substance which is able to bind to the first binding substance, wherein the second binding substance is labeled or immobilized and wherein a precipitate is formed (claims 13-15).

The references do not teach the utilization of a fusion polypeptide as a standard or a competitive antigen. However, as discussed above, Clerico et al. (1998) teach using standards of ANP and BNP in the assays for these natriuretic hormones and also teach radioactively labeled antibodies to said peptides (Figures 1 and 2); Clerico et al (2000) teach competitive radioimmunoassays (abstract) and teach the advantages of measuring the prohormones (pro-NT-ANP and pro-NT-BNP). Since, as discussed above, it would be obvious to measure both pro-ANP and pro-BNP in the same assay to increase the efficiency and reduce the costs of said assays, it would be obvious to one of skill in the art to make a fusion protein so that one could have a protein comprising equimolar amounts of pro-BNP and pro-ANP to use as a standard in immunoassays detecting both polypeptides in a single assay. One would have a reasonable expectation of success because methods of making fusion protein are well known in the art.

The '838 patent teaches methods of determining treatment regimen for use in a patient comprising determining the presence of fragments of ANP, BNP and CNP precursor peptides or fragments thereof utilizing immunoassays and correlating the presence or amount of said fragments of ANP and BNP to a disease or prognostic state (column 10, lines 53-67)

The '838 patent also teaches a sequence (SEQ ID NO:3) comprising a segment, amino acids 1-98, which is 99.4% identical to SEQ ID NO:3 of the instant invention and is identified as an ANP precursor, pro-ANP (identified as proANP 1-98 of claim 10) (pro-hormone) (See results in SCORE and alignment below).

Alignment match for SEQ ID NO:3 of the instant invention

It is noted that the one amino acid difference between the sequence as taught by the '838 patent and the instant invention is a conservative amino acid substitution of Aspartic acid for Glutamic acid; one of ordinary skill would predict that this conservative substitution would not affect the biological activity, binding characteristics, or three-dimensional configuration of the protein.

The reference also teaches a sequence, SEQ ID NO:1, comprising a segment, amino acids 1-76 which is 100% identical to SEQ ID NO:6 of the instant invention and is identified as a BNP-precursor molecule (proBNP 1-76 of claim 10) (pro-hormone) (See results in SCORE and alignment below).

Alignment match for SEQ ID NO:6 of the instant invention

Query Match 100.0%; Score 392; DB 3; Length 108;

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Best Local Similarity 100.0%; Pred. No. 4.1e-41;  
Matches 76; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

QY 1 HPLGSPGSASDLETSGLQEQRNHLQGKLSELQVEQTSLEPLQESPRTGVWKSREVATEG 60  
||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||  
Db 1 HPLGSPGSASDLETSGLQEQRNHLQGKLSELQVEQTSLEPLQESPRTGVWKSREVATEG 60

QY 61 IRGHHRKMLVYTLRAPR 76  
||||||| |||||||  
Db 61 IRGHHRKMLVYTLRAPR 76

One of ordinary skill in the art would recognize that binding substances or antibodies which recognize polypeptides comprising segments 99.4% and 100% identical to SEQ ID NO:3 and SEQ ID NO:6, respectively, of the instant invention would recognize the polypeptides of the instant invention. The '838 patent teaches measuring fragments in samples; said fragments could be pro-ANP and pro-BNP (column 15, lines 36-43). The fragments are recognized by antibodies. Said antibodies may comprise bivalent antibodies, comprising two Fab fragments linked by a disulfide bridge at the hinge region (column 16, lines 21-23), thus teaching a bispecific binding substance that binds to proANP and proBNP, as required by claims 2, 3, and 7). The antibodies may be monoclonal antibodies or polyclonal antibodies (column 16, lines 34-39), as required by claim 8. The reference teaches immunoassays comprising a tagged antibody (column 18, lines 24-26), a limitation of claim 12. The reference teaches a pure preparation of the known antigen (pro-ANP and pro-BNP, in the instant assay) is needed in order to standardize the assay (column 18, lines 35-40). The '838 patent teaches immunoassays comprising labeled anti-immunoglobulin antibodies (column 18 lines 53-55), thus meeting the limitations of claims 13 and 14. The reference also teaches "capture" or "sandwich" ELISA assays wherein the antigen-antibody-2<sup>nd</sup> antibody complex precipitates (column 18, line 60, bridging column 19, line 3) and radioimmunassays (column 18, lines 32-55).

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the methods taught by Clerico et al (1998) and Clerico et al (2000) and substitute the polypeptides of SEQ ID NOs: 3 and 1 as taught by the '838 patent for the generic proANP and pro-BNP taught by the Clerico et al

(2000) and utilize the antibodies and immunoassays taught by the '838 patent in place of the IRMA assays taught by Clerico et al (1998). The person of ordinary skill in the art would have been motivated to make these modifications because the '838 patent identifies the polypeptides of SEQ ID NOs:3 and 1 as proANP and pro-BNP and one of skill in the art would recognize that one may use bivalent antibodies to bind to different antigens, antibodies directed to the full length sequence would also bind homologous sequences or fragments of said sequences and that different types of immunoassays (RIAs, IRMAs and ELISAs) are art-recognized equivalents. Additionally, as discussed above, one would be motivated to make a fusion polypeptide, as recited in claim 10(d) comprising SEQ ID NOs:3 (proBNP 1-76) and SEQ ID NO:1 (proANP 1-98), sequences taught in the '838 patent, to use as a standard in the assays to detect both pro-ANP and pro-BNP in a single assay so that one could have a protein comprising equimolar amounts of pro-BNP and pro-ANP to use as a standard in immunoassays using bivalent antibodies. One would reasonably have expected success because the references listed above teach utilizing standards comprising ANP and BNP in the immunoassays, and methods of making fusion proteins and bivalent antibodies for use in diverse immunoassays and methods of practicing different immunoassays are well known in the art. Additionally, although the polypeptide of the prior art (SEQ ID NO:3) differs by one conservative amino acid substitution from the sequence of the claimed invention, one of ordinary skill would recognize, absent evidence to the contrary, that polypeptides comprising said sequence would have the same structural and biological characteristics (for example, binding, and antigenicity) as the polypeptide of the instant invention.

Applicants traverse the rejection (Response of 10 May 2011, page 2, 3<sup>rd</sup> paragraph, bridging page 3, 2<sup>nd</sup> paragraph). Applicants argue that the Declaration of Professors Vuolteenaho and Ruskoaho present data showing unexpected results which are compelling evidence of non-obviousness.

Applicant's arguments have been fully considered but are not found to be persuasive for reasons stated above.

***Conclusion:***

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHULAMITH H. SHAFFER whose telephone number is (571)272-3332. The examiner can normally be reached on Monday through Friday, 8 AM to 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey J. Stucker can be reached on 571-272-0911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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